55919 AR226-1067

26 pp

FYI-0202-01378

To: NCIC OPPT/DC/USEPA/US@EPA
cc: Terry OBryan/DC/USEPA/US@EPA

Subject: AR226 and FYI Submission from DuPont Haskell

Please include the attached report in both the AR226 and TSCA Section 8 FYI files. Although the cover page of the report is captioned "Trade Secret," the company has confirmed that no confidentiality provisions apply, and that the document itself contains no trade secret or TSCA Confidential Business Information.

The study title of the report is "H-24921: Dermal Sensitization Test - Buehler Method"

If you have any questions, please contact me.

Contain NO CBI

Mary F. Dominiak
U.S. Environmental Protection Agency
EPA East, Room 4410-M, Mail Code 7405M
1200 Pennsylvania Avenue, NW
Washington, DC 20460
Phone: 202-564-8104 Fax: 202-564-4775
----- Forwarded by Mary Dominiak/DC/USEPA/US on 02/21/2002 02:52 PM -----

Jill H Hogan <Jill.H.Hogan-2@usa.dupont.com>
01/15/2002 09:02 AM

To: Mary Dominiak/DC/USEPA/US@EPA
cc:
Subject: Re: Report from Gerry Kennedy

Hi Mary,

Gerry checked and it is okay to send this report to you electronically. It's a pdf file so if you have trouble opening it, please let me know.

Thanks,

Jill

(See attached file: DuPont-7977.pdf)
(See attached file: DuPont-7977.pdf)

DuPont-7977.pdf

TRADE SECRET

STUDY TITLE

H-24921: Dermal Sensitization Test - Buehler Method

LABORATORY PROJECT IDENTIFICATION

DuPont-7977

PSL Study Number 11321

Work Request Number 13890

Service Code Number 641

DATA REQUIREMENT

U.S. EPA Health Effects Test Guidelines, OPPTS 870.2600, August, 1998

AUTHOR

George E. Moore, B.S.

STUDY COMPLETED ON

December 17, 2001

PERFORMING LABORATORY

Product Safety Labs

2394 Route 130

Dayton, New Jersey, 08810

SUBMITTER

DuPont Haskell Laboratory

for Health and Environmental Sciences

Elkton Road, P.O. Box 50

Newark, Delaware 19714-0050

GOOD LABORATORY PRACTICE STATEMENT

This study meets the requirements of U.S. EPA Good Laboratory Practice Standards: Toxic Substances Control Act: 40 CFR 792 with the following exceptions:

1. Treatment solutions were not analyzed for concentration, uniformity or stability of the test and control substances. The procedures used by trained personnel to prepare the treatment solutions insured:

   a) The accuracy of concentration because the test substance diluent (vehicle) was accurately measured with a graduated device. The test substance was weighed on a balance accurate to at least two decimal places

   b) Uniformity, because all solutions were thoroughly mixed prior to administration to the test system; and

   c) Stability, because treatment solutions were prepared just prior to use.

2. The stability, uniformity of mixture and verification of concentration of HCA in its carriers were not determined.

Applicant/Sponsor: E.I. du Pont de Nemours and Company
Newark, Delaware
U.S.A.

Study Director: George E. Moore
George E. Moore, B.S.

12/17/01
Date

QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit randomly selects intervals for QA inspections prior to study initiation. Records of the findings of these inspections are kept on file. The summary below provides verification of statements made in the final report section that addresses Quality Assurance audits.

Inspections were made of:

DATE	PROCEDURE INSPECTED
10/26/01	48 hour scoring (Induction #3)
11/20/01	Raw data
11/20/01	Draft report
12/17/01	Final report

Findings reported to: Study Director 11/20/01

Management 11/20/01

Annamarie LaPorte

Annamarie LaPorte
Quality Assurance Auditor

CERTIFICATION

We the undersigned declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

George E. Moore

George E. Moore, B.S.
Study Director

Dec 17, 2001
Date

Gary Wnorowski, B.A.
Laboratory Director

Dec 17, 2001
Date

Carol Finlay

Carol Finlay, B.A.
Study Monitor

2-Jan-2002
Date

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STUDY INFORMATION

9th Collective Nomenclature:	Octanoic acid, pentadecafluoro-, ammonium salt
Synonyms/Codes:	• Ammonium perfluorooctanoate • FC-143 FLUORAD Brand Fluorochemical Surfactant (3M Chemicals) • C-8 • Perfluorooctanoate, ammonium salt • PFOA • H-24921 • Lot 332 (3M Specialty Materials) (Lot No.)
Haskell Number:	24921
CAS Registry Number:	3825-26-1
Composition:	96.5-100% Ammonium perfluorooctanoate [CAS # 3825-26-1] 0-1.5% Ammonium perfluorohexanoate [CAS # 21615-47-4] 0-1% Ammonium perfluoroheptanoate [CAS # 6130-43-4] 0-1% Heptadecafluorononanoic acid, Ammonium Salt [CAS # 4149-60-4]
Purity:	95.2%
Physical Characteristics:	White solid
Stability:	The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
Sponsor:	E. I. du Pont de Nemours and Company Wilmington, Delaware 19898 U.S.A.
Study Initiated/Completed:	September 27, 2001/ (see report cover page)
In-Life Initiated/Completed:	October 10, 2001 /November 9, 2001

H-24921: DERMAL SENSITIZATION TEST - BUEHLER METHOD

PROTOCOL NO.:	P328 DUP
AGENCY:	EPA (TSCA)
PSL STUDY NUMBER:	11321
SPONSOR:	E.I. du Pont de Nemours and Company Wilmington, Delaware 19898 U.S.A.
SUBMITTER:	DuPont Haskell Laboratory for Health and Environmental Sciences Elkton Road, P.O. Box 50 Newark, DE 19714-0050
TEST SUBSTANCE IDENTIFICATION:	H-24921
TEST SUBSTANCE DESCRIPTION:	White solid
DATE RECEIVED:	September 19, 2001
PSL REFERENCE NO.:	010919-19R
DATE OF PROTOCOL APPROVAL:	September 27, 2001
EXPERIMENTAL INITIATION DATE:	October 10, 2001
EXPERIMENTAL COMPLETION DATE:	November 9, 2001
STUDY COMPLETION DATE:	December 17, 2001
NOTEBOOK NO.:	01-55: pages 133-146

1. PURPOSE

To assess the sensitization potential of H-24921 after repeated topical applications.

2. SUMMARY

A dermal sensitization test was conducted with guinea pigs to determine the potential for H-24921 to produce sensitization after repeated topical applications.

The test substance (90%[1] w/w mixture in distilled water) was topically applied for six hours to 20 healthy test guinea pigs, once each week for a three week induction period. A test vehicle control group (ten animals) was maintained under the same environmental conditions and treated with the vehicle (distilled water, 100%) for the induction phase. Twenty-seven days after the first induction dose, a challenge dose of the test substance (HNIC, 50% w/w solution in distilled water) and vehicle (distilled water, 100%) were applied to a naive site on each of the test and test substance irritation control guinea pig. Approximately 24 and 48 hours after each induction and challenge dose, the test and test irritation control animals were scored for erythema.

A table summarizing the incidence and severity of the sensitization response noted after challenge is found below.

	Incidence with Skin Reactions[2]			
	Test Animals		Test Irritation Control Animals	
	Hours			
	24	48	24	48
50% w/w in distilled water	0/20	0/20	0/10	0/10
Distilled water	0/20	0/20	0/10	0/10

	Severity[3]			
	Test Animals		Test Irritation Control Animals	
	Hours			
	24	48	24	48
50% w/w in distilled water	0.13	0.05	0.10	0.05
Distilled water	0.00	0.00	0.00	0.00

Based on the results of this study, the test substance is considered not to be a contact sensitizer. The positive response observed in the historical positive control validation study with α-hexylcinnamaldehyde, technical grade, 85% validates the test system used in this study (See Section 7).

3. MATERIALS

A. Test Substance

The test substance identified as H-24921, was received on September 19, 2001 and was further identified with PSL Reference Number 010919-19R. The test substance was stored at room temperature. The sample was a white solid.

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[1] The test substance, as received, was a solid. To enhance skin contact, the test substance was moistened with distilled water prior to application.

[2] Animals with scores greater than 0.5

[3] Sum of the erythema scores divided by the number of animals evaluated.

Characterization of the test substance provided to Product Safety Labs by the Sponsor was:

Composition: 96.5-100% Ammonium perfluorooctanoate

pH: 5 (0.5% aqueous)

Solubility: Not applicable

Stability: The test substance is expected to be stable for the duration of testing

Expiration Date: December 15, 2001

B. Animals

3.B.1 Number of Animals: 34

3.B.2 Number of Groups: 3

3.B.3 Number of Animals per Group:
Preliminary Irritation Testing: 4
Test Group: 20
Test Vehicle Control Group: 10

3.B.4 Sex: Male

3.B.5 Species/Strain: Guinea pigs/Hartley albino

3.B.6 Age/Body weight: Preliminary Irritation Group; Young adult
Test and Test Vehicle Control Groups: Young adult/349-443 grams at experimental start

3.B.7 Source: Received from Elm Hill Breeding Labs, Chelmsford, MA on October 3, 2001

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were group housed in suspended stainless steel with mesh floors or plastic perforated bottom caging which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week.

4.A.2 Animal Room Temperature and Relative Humidity Ranges: 18-23 °C and 30-68%

4.A.3 Photoperiod: 12 hour light/dark cycle

4.A.4 Acclimation Period: 7 days

4.A.5 Food: Pelleted Purina Guinea Pig Chow #5025

4.A.6 Water: Filtered tap water was supplied *ad libitum* by automatic water dispensing system.

4.A.7 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted at least once a year and the

records are kept on file at Product Safety Labs. The dates of the most recent analyses are presented in Appendix A.

B. Identification

4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.

4.B.2 Animal: Each guinea pig was marked with a color code and given a sequential animal number assigned to study #11321, which constituted unique identification.

5. PROCEDURE

A. Preparation and Selection of Animals

Within 24 hours prior to each application, the fur of each guinea pig assigned to test and test vehicle control groups was removed by clipping the dorsal area and flanks. Care was taken to avoid abrading the skin. Prior to study initiation, the animals were weighed and the skin was checked for any abnormalities. Only healthy animals without pre-existing skin irritation were selected for test.

B. Induction Phase

Once each week for three weeks, 0.4 ml of a 90%[1] w/w mixture of the test substance in distilled water was applied to the left side of each test animal for six hours using an occlusive 25 mm Hill Top Chamber®. The chambers were secured in place and wrapped with non-allergenic Durapore™ adhesive tape to avoid dislocation of the chambers and to minimize loss of the test substance. After the six hour exposure period, the chambers were removed and the test sites were gently wiped with water and a clean towel to remove any residual test substance. Approximately 24 and 48 hours after each induction application, readings were made of local reactions (erythema) according to the scoring system described in Section 5.D. The vehicle (distilled water, 100%) was used for the induction of the test vehicle control animals and scored as above.

C. Challenge Phase

Twenty-seven days after the first induction dose, 0.4 ml of a 50% w/w solution of the test substance (HNIC) in distilled water was applied to a naive site on the right rear flank of each animal using the procedures described above. The vehicle (distilled water, 100%) was applied to the right front flank of each test animal for the challenge phase. The test substance irritation control group was also treated with the test substance and vehicle for the challenge phase. These sites were evaluated for a sensitization response (erythema) approximately 24 and 48 hours after the challenge application according to the system described in Section 5.D.

D. Scoring System

0 - no erythema
0.5 - very faint erythema, usually non-confluent
1 - faint erythema, usually confluent
2 - moderate erythema
3 - severe erythema with or without edema

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[1] The test substance, as received, was a solid. To enhance skin contact, the test substance was moistened with distilled water prior to application.

E. Body Weights

Individual body weights of the animals were recorded prior to the first induction, weekly and again on the day after challenge. The mean and standard deviation were calculated for all body weights and body weight gains. The overall mean body weight gains for test and test vehicle control animals were evaluated statistically by an unpaired t test (INSTAT Biostatistics, created by GraphPad Software, San Diego, and CA.).

F. Clinical Observations

The animals were observed daily. If any unusual clinical signs were observed, they were recorded.

6. EVALUATION

In order to evaluate the sensitization response noted during the challenge phase, two indices were used; one for incidence and one for severity. The incidence index was calculated to evaluate the incidence of erythema (sensitization response) approximately 24 and 48 hours after challenge according to the following:

Scores of 1 or greater in the test group are required to be indicative of sensitization. If scores of one (1) or greater are seen on the control animals, then the reactions of the test substance group animals that exceed the most severe control reactions are considered to be positive scores.

Incidence is reported as the number of positive animals in each group divided by the total number of animals tested in that group.

Severity is reported as the sum of the test grades divided by the total number of animals tested in a given group determined for both 24 and 48 hours. All average grades are to be rounded off to the nearest tenth of a unit.

7. HISTORICAL POSITIVE CONTROL VALIDATION STUDY

The procedures used in this study were validated using α-hexylcinnamaldehyde, technical grade, 85% (purity) as a positive control substance. The most recent validation, PSL Study #10548, was performed by Product Safety Labs and completed on May 10, 2001. The raw data and report for this study are archived in Product Safety Labs Historical Data Notebook No. 01, pages 20-28. This test was conducted at the Dayton Facility with HCA using Hartley strain albino guinea pigs from Elm Hill Breeding Labs following induction and challenge procedures similar to those described in Section 5. The data from this positive control study is summarized below.

	Sensitization Response Indices			
	Incidence of Positive Response[1]		Severity[2]	
	Hours			
	24	48	24	48
Positive Control Animals	5/10	2/10	0.70	0.40
Positive Vehicle Control Animals	0/5	0/5	0.45	0.30

[1] Animals with scores greater than 0.5.

[2] Sum of the erythema scores divided by the number of animals evaluated.

8. STUDY CONDUCT

This study was conducted at Product Safety Labs, 2394 Route 130, Dayton, New Jersey, 08810 in compliance with the following regulation:

U.S. EPA Good Laboratory Practice Standards: Toxic Substances Control Act (TSCA): 40 CFR 792

The procedures as described in the protocol are based on the following testing guideline:

U.S. EPA Health Effects Test Guidelines, OPPTS 870.2600, August, 1998

The primary technician for this study was Rolland Colis, B.S.

9. REFERENCES

Robinson, M., Nusair, T., Fletcher, E., and Ritz, H., A Review of the Buehler Guinea Pig Skin Sensitization Test And Its Use in a Risk Assessment Process for Human Skin Sensitization in *Toxicology*, 61, 91-107, 1990.

Ritz, H., and Buehler, E., Planning, conduct, and interpretation of guinea pig sensitization patch tests, in *Current Concepts in Cutaneous Toxicity*, V.A. Drill and P. Lazar (Eds.), Academic Press, New York, 25, 1980.

10. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol and Product Safety Labs Standard Operating Procedures. Dates of inspections and audits performed during the study, and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

11. DEVIATION FROM THE FINAL PROTOCOL

None

12. FINAL REPORT AND RECORDS RETENTION

A copy of the signed report, copies of all raw data generated at Product Safety Labs and a copy of the original signed protocol, will be maintained in the Product Safety Labs archives.

Laboratory-specific or site-specific raw data, such as personnel files and equipment records will be retained by the facility where the work was done.

The original raw data, the original final report and a copy of the protocol will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

13. RESULTS

Preliminary irritation testing scores for the test substance and historical positive control animals are presented in Table 1. Individual body weights and body weight gain for test and historical positive control animals are presented in Tables 2 and 3, respectively. Induction and Challenge Phase skin reaction scores for test and historical positive control animals are presented in Tables 4 through 7.

All test and control animals survived and appeared normal throughout the study. There were no statistically significant differences between the overall body weight gain of the test and test vehicle control animals.

Induction Phase

Test Animals (test substance applied as a 90%[1] w/w mixture in distilled water): Very faint to faint erythema (0.5-1) was noted for all test sites during the induction phase.

Test Vehicle Control Animals (distilled water, 100%): No dermal irritation was noted for any test vehicle control site during the induction phase.

Historical Positive Control Animals (HCA, as received): Very faint to faint erythema (0.5-1) was noted for all test sites during the induction phase.

Challenge Phase

Test Animals (test substance applied as a 50% w/w solution in distilled water): Very faint erythema (0.5) was noted for five of 20 test sites 24 hours after challenge. Irritation persisted at two of these sites through 48 hours.

Test Substance Irritation Control Animals (test substance applied as a 50% w/w solution in distilled water): Very faint erythema (0.5) was noted for two of ten test sites 24 hours after challenge. Irritation persisted at one of these sites through 48 hours.

Test Animals (distilled water, 100%): No dermal irritation was noted for any test site following the challenge phase.

Test Substance Irritation Control Animals (distilled water, 100%): No dermal irritation was noted for any test substance irritation control site following the challenge phase.

Historical Positive Control Animals (applied as a 75% w/w solution of HCA (as received) in mineral oil): Five of ten animals exhibited signs of a sensitization response (faint erythema [1]) 24 hours after challenge. Similar indications persisted at two of these sites with very faint erythema (0.5) noted for most other sites through 48 hours.

Historical Positive Vehicle Control Animals (applied as a 75% w/w solution of HCA (as received) in mineral oil): Very faint erythema was noted for four of five test naive control sites 24 hours after challenge. Irritation persisted at three of these sites through 48 hours.

14. CONCLUSION

Based on these findings and on the evaluation system used, H-24921 is considered not to be a contact sensitizer.

The positive response observed in the historical positive control validation study with α-hexylcinnamaldehyde, technical grade, 85% (as received) validates the test system used in this study (See Section 7).

---

[1] The test substance, as received, was a solid. To enhance skin contact, the test substance was moistened with distilled water prior to application.

TABLE 1: PRELIMINARY IRRITATION TESTING SCORES FOR DETERMINATION OF HNIC[1] (TEST SUBSTANCE)

Animal No.	Sex	CONCENTRATION (%)[2]							
		90[3]		75		50		25	
		Hours after Patch Removal							
		24	48	24	48	24	48	24	48
2998	M	0.5	0.5	0.5	0.5	0.5	0	0	0
2999	M	0.5	0.5	0.5	0.5	0	0	0	0
3000	M	0.5	0.5	0.5	0	0	0	0	0
3001	M	1	1	1	1	0.5	0.5	0	0

[1] HNIC - Highest Non-Irritating Concentration

[2] Four-tenths of a milliliter of the test substance was applied as w/w mixtures, suspensions or solutions in distilled water using an occlusive 25 mm Hill Top Chamber®.

[3] The test substance, as received, was a solid. To enhance skin contact, the test substance was moistened with distilled water prior to application.

TABLE 1 (cont.): PRELIMINARY IRRITATION TESTING SCORES FOR DETERMINATION OF HNIC[1] (POSITIVE CONTROL-HCA)

Historical Positive Control Validation Study

Animal No.	Sex	Concentration (%)[2]			
		100	75	50	25
0210	M	0.5	0.5	0	0
0211	M	0.5	0	0	0
0212	M	0.5	0	0	0
0213	M	0.5	0.5	0	0

[1] HNIC - Highest Non-Irritating Concentration

[2] Four-tenths of a milliliter of HCA was applied as received and as w/w solutions in mineral oil using an occlusive 25 mm Hill Top Chamber®.

TABLE 2: INDIVIDUAL BODY WEIGHTS/WEIGHT GAIN (g)

Test Substance Group

Animal No.	Sex	Initial	Week 2	Gain (0 to wk 2)	Week 3	Gain (wk 2 to 3)	Week 4	Gain (wk 3 to 4)	Day After Challenge	Gain (wk 4 to 5)	Total Weight Gain (wk 0 to 5)
3148	M	419	459	40	536	77	594	58	652	58	233
3149	M	371	403	32	471	68	516	45	567	51	196
3150	M	385	421	36	485	64	525	40	567	42	182
3151	M	381	413	32	464	51	525	61	562	37	181
3152	M	443	484	41	552	68	628	76	664	36	221
3153	M	427	482	55	548	66	615	67	681	66	254
3154	M	389	428	39	493	65	553	60	603	50	214
3155	M	375	422	47	481	59	536	55	586	50	211
3156	M	352	405	53	463	58	516	53	566	50	214
3157	M	396	458	62	527	69	589	62	647	58	251
3158	M	372	395	23	444	49	482	38	532	50	160
3159	M	373	413	40	463	50	514	51	552	38	179
3160	M	404	471	67	527	56	592	65	628	36	224
3161	M	381	443	62	505	62	554	49	596	42	215
3162	M	396	434	38	473	39	537	64	566	29	170
3163	M	421	472	51	539	67	601	62	668	67	247
3164	M	388	429	41	491	62	531	40	584	53	196
3165	M	404	444	40	511	67	544	33	595	51	191
3166	M	374	444	70	516	72	582	66	653	71	279
3167	M	394	458	64	490	32	526	36	581	55	187
Mean		396.2	449.4	53.2	509.4	60.0	556.8	47.4	616.2	59.4	220
Standard Deviation		17.61	16.27	13.48	20.23	16.05	33.04	15.42	41.12	8.99	40.98

TABLE 2 (cont.): INDIVIDUAL BODY WEIGHTS/WEIGHT GAIN (g)

Test Vehicle Control Group

Animal No.	Sex	Initial	Week 2	Gain (0 to wk 2)	Week 3	Gain (wk 2 to 3)	Week 4	Gain (wk 3 to 4)	Day After Challenge	Gain (wk 4 to 5)	Total Weight Gain (wk 0 to 5)
3168	M	392	434	42	502	68	547	45	603	56	211
3169	M	398	439	41	497	58	553	56	616	53	218
3170	M	385	419	34	482	63	540	58	590	50	205
3171	M	401	452	51	522	70	581	59	585	4	184
3172	M	381	409	28	459	50	503	44	542	39	161
3173	M	381	439	58	497	58	566	69	606	40	225
3174	M	368	408	40	456	48	502	46	533	31	165
3175	M	382	431	49	484	53	540	56	577	37	195
3176	M	349	409	60	479	70	526	47	583	57	234
3177	M	398	461	63	531	70	592	61	633	41	235
Mean		375.6	429.6	54.0	489.4	59.8	545.2	55.8	586.4	41.2	210.8
Standard Deviation		18.28	22.18	9.41	27.57	9.96	34.95	9.68	37.11	9.65	30.30

TABLE 3: INDIVIDUAL BODY WEIGHTS/WEIGHT GAIN (g)

Historical Positive Control Validation Study[1]

Animal No.	Sex	Initial	Week 2	Gain (0 to wk 2)	Week 3	Gain (wk 2 to 3)	Week 4	Gain (wk 3 to 4)	Day After Challenge	Gain (wk 4 to 5)	Total Weight Gain (wk 0 to 5)
277	M	396	448	52	508	60	550	42	588	38	192
278	M	407	470	63	535	65	610	75	619	9	212
279	M	397	456	59	512	56	565	53	598	33	201
280	M	393	452	59	496	44	558	62	624	66	231
281	M	407	466	59	512	46	585	73	639	54	232
282	M	396	444	48	490	46	549	59	613	64	217
283	M	392	473	81	524	51	590	66	630	40	238
284	M	357	426	69	465	39	540	75	597	57	240
285	M	399	472	73	511	39	561	50	625	64	226
286	M	364	430	66	473	43	519	46	588	69	224
Mean		390.8	453.7	62.9	502.6	48.9	562.7	60.1	612.1	49.4	221.3
Standard Deviation		16.84	16.96	9.79	21.80	8.88	26.42	12.15	18.22	19.10	15.78
Vehicle Control											
287	M	367	424	57	484	60	540	56	588	48	221
288	M	374	431	57	480	49	547	67	598	51	224
289	M	359	421	62	499	78	559	60	619	60	260
290	M	386	453	67	506	53	554	48	624	70	238
291	M	411	479	68	543	64	598	55	664	66	253
Mean		379.4	441.6	62.2	502.4	60.8	559.6	57.2	618.6	59	239.2
Standard Deviation		20.26	24.37	5.26	25.07	11.26	22.63	6.98	29.37	9.43	17.22

[1] PSL Study #10548, performed by PSL and completed on May 10, 2001.

TABLE 4: INDUCTION PHASE SKIN REACTION SCORES

Test Substance Group[1]

Induction Number	1		2		3	
Hours[2]	24	48	24	48	24	48
Animal No.						
3148	0.5	0.5	0.5	0	0.5	0
3149	0.5	0.5	0.5	0.5	0.5	0.5
3150	0.5	0	0.5	0	0.5	0
3151	1	0.5	0.5	0	0.5	0
3152	0	0	0.5	0.5	0.5	0
3153	0.5	0.5	0.5	0	1	0.5
3154	0	0	0.5	0	0.5	0.5
3155	0.5	0	0.5	0.5	0.5	0.5
3156	0.5	0	1	0.5	0.5	0.5
3157	0.5	0.5	0.5	0	0.5	0
3158	0.5	0.5	0.5	0	0.5	0
3159	0.5	0	1	0.5	0.5	0.5
3160	0	0	0.5	0.5	0.5	0
3161	0	0	0.5	0	0.5	0
3162	1	1	0.5	0	0.5	0.5
3163	0.5	0.5	0.5	0.5	0.5	0
3164	1	0.5	1	0.5	1	0.5
3165	0.5	0.5	0.5	0	1	0.5
3166	0.5	0.5	0.5	0	0.5	0
3167	0.5	0	0.5	0.5	0.5	0.5

[1]Four-tenths of a milliliter of the test substance was applied as a 90% w/w mixture in distilled water, using an occlusive 25 mm Hill Top Chamber®.

[2] Hours after induction dose.

TABLE 4 (cont.): INDUCTION PHASE SKIN REACTION SCORES

Test Vehicle Control Group[1]

Induction Number	1		2		3	
Hours[2]	24	48	24	48	24	48
Animal No.						
3168	0	0	0	0	0	0
3169	0	0	0	0	0	0
3170	0	0	0	0	0	0
3171	0	0	0	0	0	0
3172	0	0	0	0	0	0
3173	0	0	0	0	0	0
3174	0	0	0	0	0	0
3175	0	0	0	0	0	0
3176	0	0	0	0	0	0
3177	0	0	0	0	0	0

[1] Four-tenths of a milliliter of the vehicle (distilled water, 100%), was applied using an occlusive 25 mm Hill Top Chamber®.

[2] Hours after induction dose.

TABLE 5: INDUCTION PHASE SKIN REACTION SCORES

Historical Positive Control Validation Study[1]

Positive Control Group[2]

Induction Number	1		2		3	
Hours[3]	24	48	24	48	24	48
Animal No.						
277	0.5	0	0.5	0	0.5	0
278	0.5	0.5	1	0.5	1	0.5
279	0.5	0.5	0.5	0	1	0.5
280	0	0	0.5	0	0.5	0.5
281	0.5	0.5	0.5	0.5	1	0.5
282	0.5	0	0.5	0	1	0.5
283	0	0	0.5	0	0.5	0.5
284	0.5	0	0.5	0	1	0.5
285	0.5	0.5	0.5	0.5	1	0.5
286	0	0	0.5	0	0.5	0

[1] PSL Study #10548, performed by PSL and completed on May 10, 2001.

[2] Four-tenths of a milliliter of HCA was applied as received using an occlusive 25 mm Hill Top Chamber®.

[3] Hours after induction dose.

TABLE 6: CHALLENGE PHASE SKIN REACTION SCORES

Test Substance Group[1]

Animal No.	Hours after Dosing			
	24		48	
	50%	Distilled water (100%)	50%	Distilled water (100%)
3148	0	0	0	0
3149	0	0	0	0
3150	0	0	0	0
3151	0	0	0	0
3152	0	0	0	0
3153	0.5	0	0	0
3154	0.5	0	0	0
3155	0	0	0	0
3156	0	0	0	0
3157	0	0	0	0
3158	0.5	0	0.5	0
3159	0	0	0	0
3160	0	0	0	0
3161	0.5	0	0	0
3162	0.5	0	0.5	0
3163	0	0	0	0
3164	0	0	0	0
3165	0	0	0	0
3166	0	0	0	0
3167	0	0	0	0

[1] Four-tenths of a milliliter of the test substance was applied as a 50% w/w solution in distilled water, using an occlusive 25 mm Hill Top Chamber® to the right rear flank and the vehicle (distilled water, 100%) was applied to the right front flank.

TABLE 6 (cont.): CHALLENGE PHASE SKIN REACTION SCORES

Test Substance Irritation Control Group[1]

Animal No.	Hours after Dosing			
	24		48	
	50%	Distilled water (100%)	50%	Distilled water (100%)
3168	0.5	0	0	0
3169	0	0	0	0
3170	0	0	0	0
3171	0	0	0	0
3172	0.5	0	0.5	0
3173	0	0	0	0
3174	0	0	0	0
3175	0	0	0	0
3176	0	0	0	0
3177	0	0	0	0

[1] Four-tenths of a milliliter of the test substance was applied as a 50% w/w solution in distilled water, using an occlusive 25 mm Hill Top Chamber® to the right rear flank and the vehicle (distilled water, 100%) was applied to the right front flank.

TABLE 7: CHALLENGE PHASE SKIN REACTION SCORES[1]

Historical Positive Control Validation Study[2]

Animal No.	Hours[3]	
Positive Control	**24**	**48**
277	1	0.5
278	0.5	0.5
279	0.5	0.5
280	1	1
281	1	0.5
282	1	1
283	0.5	0
284	0.5	0
285	1	0.5
286	0	0
Vehicle Control		
287	0.5	0.5
288	0	0
289	0.5	0.5
290	0.5	0.5
291	0.5	0

[1] Four-tenths of a milliliter of a 75% w/w solution of HCA (as received) in mineral oil using an occlusive 25 mm Hill Top Chamber®.

[2] PSL Study #10548, performed by PSL and completed on May 10, 2001.

[3] Hours after challenge dose.

APPENDIX A: FEED AND WATER ANALYSES

On June 21, 2001 animal feed was analyzed for the presence of the following contaminants:

Aldrin	Dylox (Trichlorfon)
BHC-A (Alpha-Hexachlorocyclohexane)	Endosulfan I & II
BHC-B (Beta-Hexachlorocyclohexane)	Endsolfan Sulfate
BHC-D (Delta-Hexachlorocyclohexane)	Endrin
BHC-G (Lindane)	Endrin Aldehyde
Captan	Esfenvalerate
Chlordane	Fenvalerate
Chlorpyrifos-Methyl	Heptachlor
Chlorpyrifos (Dursban)	Heptachlor Epoxide
4,4 DDD	Mavrik (Tau-Fluvalinate)
4,4 DDE	Methoxychlor
4,4 DDT	Mirex
Dieldrin	Quintozene

None of the above compounds were present above the limit of detection (0.005 ppm)

LABORATORY: FOOD PRODUCTS LABORATORY, INC.
12003 N.E. Ainsworth Circle
Suite 105
Portland, OR 97220

On June 21, 2001, water was analyzed for NJDEPE Safe Drinking Water Act parameters.

LABORATORIES:

NEW JERSEY LABORATORIES
NJDEPE LAB I.D. #15001
A.A. Labs Division
222 Easton Avenue
New Brunswick, NJ 08901

SILLIKER LABORATORIES
OF NEW JERSEY, INC.
400 South Avenue
Garwood, NJ 07027

Results of water analysis for possible contaminants were acceptable within regulatory standards.